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(57) Abstract

A method for activating a mammalian immune system entails a series of continuous IL-2 infusions that are effected intermittently over an extended period. For exemple, IL-2 can be administered continuously for a period that is on the order of 5 days in length, and successive infusions of this nature can be separated by a period of at least 4 weeks. Sustained beneficial effects, including elevated CD4 cell counts, restoration of lymphocyte function and an increase in the number of IL-2 receptors, are achieved with such intermittent IL-2 therapy, which can be combined with another therapy which targets a specific disease state, such as an anti-retroviral therapy comprising, for example, the administration of AZT, ddI or interferon alpha. In addition, IL-2 administration can be employed to facilitate *in situ* transduction of T cells in the context of gene therapy. By this approach the cells are first activated *in vivo* via the aforementioned IL-2 therapy, and transduction then is effected by delivering a genetically engineered retroviral vector directly to the patient.

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IMMUNOLOGIC ENHANCEMENT WITH INTERMITTENT INTERLEUKIN-2 THERAPY

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Background of the Invention

The present invention pertains to a method for activating the immune system of a patient by intermittently administering interleukin-2 (IL-2) to that patient. Such administration of IL-2 can optionally be combined with other therapies, *e.g.*, anti-retroviral, anti-bacterial, or anti-fungal therapies, suitable for treatment of the patient's condition. This invention also relates to an approach to gene therapy that entails the use of IL-2 given to a patient so as to facilitate *in situ* lymphocyte transduction by a retroviral vector also administered to the same patient.

Attempts at immune activation and restoration in the past have utilized bone marrow transplantation or lymphocyte transfers (H.C. Lane *et al.*, *Ann. Internal Med. 113*: 512-19 (1990)), immunomodulating agents such as immuthiol (J.M. Lang *et al.*, *Lancet 24*: 702-06 (1988)) or isoprinosine (C. Pedersen *et al.*, *N. Engl. J. Med. 322*: 1757-63 (1990)), and recombinant cytokines such as interferon alpha (IFN-α) (H.C. Lane *et al.*, *Ann. Intern. Med. 112*: 805-11 (1990)) or IL-2. H.C. Lane *et al.*, *J. Biol. Response Mod. 3*, 512-16 (1984); D.H. Schwartz *et al.*, *J. Acquir. Immune Defic. Syndr. 4*, 11-23 (1991); P. Mazza *et al.*, *Eur. J. Haematol. 49*: 1-6 (1992); H.W. Murray *et al.*, *Am. J. Med. 93*: 234 (1992); H. Teppler *et al.*, *J. Infect. Dis. 167*: 291-98 (1993); P. Volberding *et*

al., AIDS Res. Hum. Retroviruses 3: 115-24 (1987) These studies have resulted in minimal or transient immune system restoration.

The use of biologic response modifiers in general, and of IL-2 in particular, is an active area of clinical research. Interleukin-2 is a T cell-derived lymphokine with a number of immunomodulating effects including activation, as well as induction of proliferation and differentiation, of both T and B lymphocytes (K.A. Smith, *Science* 140: 1169-76 (1988)). Exogenous IL-2 has been shown *in vitro* to increase the depressed natural killer cell activity and cytomegalovirus-specific cytotoxicity of peripheral blood mononuclear cells from patients with AIDS (A.H. Rook *et al., J. Clin. Invest. 72*: 398-403 (1983)), as well as to increase IFN-γ production by lymphocytes from patients with AIDS (H.W. Murray *et al., loc. cit. 76*: 1959-64 (1985)).

Il-2 given by high dose infusion has been employed in the treatment of renal cell carcinoma and melanoma (*J. Nat'l Cancer Inst.* 85(8): 622-32 (1993)). For example, doses of 36 million international units (MU) given continuously over a period of 24 hours has been used in the treatment of cancer (18 MU is equivalent to about 1mg protein). See *Lancet* 340: 241 (1992). The use of high doses of IL-2 generally is not well tolerated by patients, however, and side effects are more pronounced at such high levels.

Other researchers are evaluating IL-2 in the treatment of other diseases, including HIV infection. The use of lower doses of IL-2 in a continuous therapy regime has been disclosed by Yarchoan *et al.*, U.S. patent No. 5,026,687. More specifically, Yarchoan *et al.* teach the use of the anti-retroviral agent ddI in combination with IL-2 administered continuously at a dosage between 25,000 to 1 million international units (U) per day, for a period of three months. While Yarchoan *et al.* predict that "beneficial results" will accompany the combined ddI/IL-2 regimen, they do not attribute these results to

IL-2 *per se*. Moreover, although dosages at this lower level have been shown to cause initial increases in CD4 levels, these increases were transient in nature, *i.e.*, CD4 levels returned to baseline within 6 months after the completion of the treatment.

Many researchers feel that the use of IL-2 is contraindicated in patients with HIV infection due to its potential to activate HIV. No method of treatment of HIV with IL-2 has been disclosed which results in a sustained response, or which yields long-term beneficial results.

Cells that have been stimulated to actively synthesize DNA are susceptible to transduction by gene transfer therapy. Present methods of gene therapy require a complicated, *in vitro* transformation. More specifically, cells are removed from a patient, activated *in vitro*, and used to establish cell lines which are then gene-transduced *in vitro* and reimplanted in the patient. This procedure is expensive, and its success its limited due to the potential of failure at each of the steps of activating the cells, effecting the transduction, and implanting the cells in the patient for expression.

Attempts at using retroviral vectors to effect *in vivo* gene transfer have been limited. Retroviruses will only integrate stably into target cells that are actively synthesizing DNA. This integration must occur before retroviral gene expression can be effected. Because only a fraction of cells are actively producing DNA at any giving time, such *in vivo* gene transfer methods have shown little success.

Accordingly, it is apparent that a need exists for means to enhance a patient's immune system which is compatible with anti-viral, anti-bacterial or anti-fungal therapies.

Summary of the Invention

It is therefore an object of the present invention to provide a means for activating the immune system that

employs IL-2 but that avoids the pronounced side-effects associated with conventional IL-2 treatments.

It is also an object of the present invention to provide a means for treating a wide variety of disease states, including HIV infection, through the use of IL-2 therapy.

It is a further object of the present invention to provide an approach to effecting retroviral vector-mediated transduction *in situ*, in the context of gene therapy, for a patient whose immune system has been activated by the administration of IL-2.

In accomplishing these and other objects, there has been provided, in accordance with one aspect of the present invention, the use of an amount of IL-2 in the preparation of an agent for use in a method for treating a disease state characterized by an immunological impairment, where

(A) the amount of IL-2 is sufficient to increase the level of helper/inducer T-cell function in a patient suffering such an impairment, and

(B) the patient is administered the IL-2 in a series of infusions affected intermittently, each of the infusions being continuous over a period of time from 1 day to 2 weeks, and successive infusions being separated by a period of at least 4 weeks.

An increase in T-cell function may be gauged, for example, by an increased CD4 T-cell function in the patient, or by an increased expression of IL-2 receptors in the patient.

In accordance with another aspect of the present invention, a composition of matter has been provided for administration of IL-2. This composition comprises (i) a container suitable for holding a solution to be infused into a patient, (ii) a liquid preparation comprising an amount of IL-2 in a pharmaceutically acceptable carrier such that the preparation represents an IL-2 dosage of between about 1.8 to about 24 MU, and (iii) instructions on infusing a patient with the

preparation, said patient suffering an immunological impairment or infectious disease, such that the patient receives a continuous infusion of the dosage over a period of approximately five days.

In a preferred embodiment, the composition further comprises instructions that further direct administering a therapy to the patient prior to or concomitantly with the infusing, wherein the therapy targets a specific disease state. In another preferred embodiment, the instructions direct the administration of anti-retroviral therapy, such as the administration of zidovudine.

In accordance with yet another aspect of the present invention, a composition of matter has been provided for administration of IL-2 and a retroviral vector. This composition comprises (i) a container suitable for holding a solution to be infused into a patient, (ii) a liquid preparation comprising an amount of IL-2 in a pharmaceutically acceptable carrier such that the preparation represents an IL-2 dosage of between about 1.8 to about 24 MU, and (iii) instructions on infusing a patient with the preparation, such that the immune system of the patient is activated, and administering to the patient a retroviral vector to effect *in situ* transformation of lymphocytes. The composition may be administered at the appropriate time during the period of IL-2 infusion, such as on the 4th to 7th day of IL-2 infusion.

Additional objects and advantages of the invention will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages may be realized and obtained by means of the uses and compositions particularly pointed out in the appended claims.

Brief Description of the Drawings

Figures 1A and 1B show changes in CD4 cell count and blastogenic responses to tetanus toxoid and pokeweed mitogen (PWM) for patients 1 and 3 during a year of intermittent IL-2 therapy.

Figure 2A shows changes in lymphocyte cell surface expression of IL-2 receptors (CD25) and human leukocyte antigen-D related (HLA-DR) expression for patient 2 during a year of IL-2 therapy.

Figure 2B shows a two-color fluorescent activated cell sorter (FACS) analysis of IL-2 receptor and HLA-DR expression determined on frozen cells of patient 2 obtained prior to IL-2 therapy, and at week 48 (five weeks after the fifth course of IL-2).

Figure 3 shows changes in viral markers during IL-2 therapy for patients 2, 3, 4, 6 and 8.

Figure 4 shows levels of DNA synthesis occurring *in vivo* in patients receiving a 5-day continuous infusion of IL-2.

Figure 5 shows changes in T cell count for patients receiving anti-retroviral therapy alone (Group B) or anti-retroviral therapy and intermittent IL-2 therapy (Group A).

Figure 6 shows changes in T cell count for a patient with idiopathic CD4 lymphopenia who received intermittent IL-2 therapy.

Detailed Description of the Preferred Embodiments

The present invention provides a use of an amount of IL-2 in the preparation of an agent for use in a method to increase the level of immune function of patients, including immunosuppressed patients. The increase in immune function typically manifests itself as an increase in helper/inducer T-cell function. More particularly, the increased immune function can include, for example, an increase in CD4 count, a restoration of lymphocyte

function and/or an increase in the expression of IL-2 receptors (IL-2r).

The use of an IL-2 agent within the present invention can be effective against disease states in which IL-2 plays a role in the associated immune response. The targeted disease state can comprise, for instance, an infection of the patient by a pathogen against which a cellular immune response is the principal mechanism for specific immunity therefor in the patient, such as viral infections. See Abbas *et al.*, CELLULAR AND MOLECULAR IMMUNOLOGY 309-10 (W.B. Saunders Co., Philadelphia 1991). Illustrative of specific disease states in treatment of which the present invention can be applied are HIV infection and other diseases characterized by a decrease of T-cell immunity, for example, mycobacterial infections like tuberculosis and fungal infections such as cryptococcal disease. This method can also be used in the treatment of secondary infections that occur in patients with suppressed immune systems, such as the opportunistic infections that occur in AIDS patients.

While prior attempts at the therapeutic use of IL-2 in treating AIDS patients have been largely unsuccessful, the therapeutic use of IL-2 according to the present invention elicits maximal T-cell activation and T-cell expansion in patients with HIV infection, and should be effective in a qualitatively similar manner in any patient. The method promotes at least partial restoration of immune function of HIV-infected patients, as demonstrated by sustained improvements in CD4 counts as well as by restoration of T-cell responsiveness to recall antigens and mitogens, with results sustained up to four months after IL-2 infusion has been stopped. CD4 levels have been restored to and sustained at levels seen in healthy patients (800-1200 cells/mm^3)or even higher, indicating a restoration of the immune system as a result of the IL-2 therapy.

The present invention utilizes multiple, continuous infusions of IL-2, where the infusions are administered

intermittently over an extended period of time. The IL-2 is administered by continuous infusion which can be through a central line, *i.e.*, through the neck, or peripherally, *e.g.*, through the arm. Advantageously, the continuous IL-2 infusion can be administered peripherally. By contrast, previously disclosed low-dose continuous IL-2 treatments require central line infusions, which cause more discomfort to the patient. In addition, we anticipate that similar results will be seen with intermittent subcutaneous administration of IL-2.

The dosages of IL-2 which are characteristic of the present invention range from 1 million international units (MU)/day to 24 MU/day. These doses are much higher than doses previously used in the treatment of AIDS, but lower than those used in the treatment of cancer. In a preferred embodiment, IL-2 is administered by continuous IV infusion over 5 days, once every 8 weeks, at doses between about 6 to 18 million international units (MU)/day. Patients have been observed to show initial increases in expression of IL-2 receptors after 2 to 4 courses of this therapy. Although a dosage of 18 MU/day is preferred, some patients may not be able to tolerate this high level of IL-2, and dosages of 6-12 MU/day may be used with benefit.

According to the present invention, an IL-2 agent is administered intermittently. In particular, the IL-2 agent is administered continuously for period of time of from 1 day to 2 weeks. Infusion periods of less than one day will not be effective, and infusion periods of longer than 2 weeks seem to show no advantage over shorter periods. Studies have shown that peak activation of the immune system occurs at about the 5th day of IL-2 infusion, so an infusion period of about 1 week is preferred.

The time period between successive infusions can vary from 4 weeks to six months, or even one year. Infusions closer than 4 weeks apart are too close to yield the

benefits of intermittent therapy, and would not be beneficial to the patient. With infusions closer than 4 weeks apart, the dosages of the present invention are not well tolerated. In light of the side effects associated with IL-2 therapy, and the need to be hospitalized for the continuous infusion treatments, longer time periods between infusions are preferred. For example, the IL-2 can be administered every 6 weeks, 8 weeks, 12 weeks, or six months, and beneficial results may be seen. It is hoped that treatments as far apart as one year or longer would show sustained beneficial results.

An optimal time period between infusions has not been determined, and would probably vary from patient to patient. One skilled in the art would be able to modify a protocol within the present invention, in accordance with conventional clinical practice, to obtain optimal results for a given patient. For example, in one study patients were continuously infused with IL-2 at the above described dosages for 5 days, no IL-2 was given for 8 weeks, and then IL-2 was again given continuously for 5 days. The cycle is continued, and patients have undergone 3-7 courses of IL-2.

The intermittent IL-2 therapy of this invention can constitute a lifelong treatment regime, with the cycles of IL-2 infusions continuing indefinitely. It is believed that once a patient's immune system has been restored by this method, as evidenced by sustained CD4 counts at or above normal levels, subsequent infusions can be administered further and further apart. For example, a patient initially receiving infusions every 8 weeks may subsequently receive infusions every 6 months, and then once a year, and still maintain elevated CD4 counts.

The intermittent administration of IL-2 may be analogous to the *in vitro* approach of alternating cycles of stimulation with rest needed for the establishment or expression of T-cell lines or clones (M. Kimoto & G.G. Fathman, *J. Exp. Med. 152*: 759-70 (1980)). IL-2 could

possibly also prolong T-cell survival by altering HIV-envelope mediated programmed cell death, which may play a role in CD4 depletion in HIV infection. D.I. Cohen *et al., Science 156*: 542-45 (1992); H. Groux *et al., J. Exp. Med. 175*: 331-40 (1992) Additionally, IL-2 may be serving to alter the balance between Th1 and Th2 lymphocytes, and thus reverse the relative deficiency of Th1 cells that has recently been suggested to occur in HIV infection (H.C. Lane *et al., N. Engl. J. Med.* (1984); M. Clerici *et al., J. Clin. Invest. 91*: 759-65 (1993)).

Present studies have focused on treating HIV-infected patients with a relatively intact immune system. The degree of response of the patient to the treatment has been shown to be directly correlated to the level of immune system remaining in the patient, or inversely related to the level of the virus in the patient. The degree of remaining immune system can be measured by the T4 or CD4 count of the patient. Patients with a T4 or CD4 count above about 150 cells/mm^3 were found to respond well to the method of treatment of the present invention. The level of virus in the patient can be measured by viral titer. If plasma diluted at a ratio of about 1:81 is able to grow virus, the patient is not recommended for immediate treatment without concomitant anti-retroviral therapy.

Patients with viral levels too high can be first treated with ddI, AZT, or other anti-retroviral drugs to lower the viral burden. Alternatively, the anti-retroviral therapy can be administered simultaneously with the IL-2 therapy, allowing patients with weaker immune systems and higher viral burdens to benefit from the intermittent IL-2 therapy.

Another reason for administering concomitant anti-retroviral therapy to AIDS patients undergoing IL-2 therapy is the major concern that the viral burden of HIV patients receiving IL-2 therapy will be increased, since HIV replicates more readily in activated cells. To minimize the possible effects of increased viral burden,

the IL-2 therapy is preferably combined with an anti-retroviral therapy. Such anti-retroviral therapy can comprise, for example, the administration of AZT, AZT and ddI, or interferon alpha. The anti-retroviral therapy can commence before the IL-2 therapy is started, and can continue throughout the course of the intermittent IL-2 therapy. When patients are receiving concomitant anti-retroviral therapy, it appears that increased viral replication occurs only in the brief interval around the infusion of IL-2. In this setting, potent agents, for example, U-90152 (Upjohn), PMEA (Gilead), and CD4-PE (Upjohn), may be used intermittently for short periods of time without the development of resistant strains of virus.

When the intermittent IL-2 therapy of the present invention is used in the treatment of disease states other than HIV infection, additional therapies which target such disease states also can be used in conjunction with the IL-2 therapy. For example, anti-bacterial agents could be used in the treatment of bacterial infections and anti-fungal agents could be used in the treatment of fungal conditions. As disclosed above with reference to anti-retroviral therapy, such treatments could be used prior to or concomitant with the intermittent IL-2 therapy of the present invention.

Progress achieved by IL-2 therapy within the present invention can be measured by many parameters. The IL-2 agent of the present invention boosts the helper/inducer T-cell function of the cells. The helper T-cells activate various T effector cells that generate cell-mediated responses to antigens, including an increased production of IL-2 and IL-2 receptors. See J. Kuby, IMMUNOLOGY 17-18 (W.H. Freeman and Co., New York 1992). The increase in IL-2 receptors observed in patients undergoing therapy by this method is consistent with such an elevation of helper/inducer T-cell function.

Studies have shown that in HIV-infected patients, responses of peripheral blood lymphocytes, as measured by

lymphocyte blast transformation as well as by IL-2 production, tend to be lost initially to recall antigens, then to alloantigens, and finally, as immunosuppression becomes severe, to mitogens such as phytohemagglutinin and pokeweed mitogen (M.T. Lotze *et al., Cancer 58*: 2754-2772 (1986); H.C. Lane *et al., New England J. Med.* 313: 79-84 (1985); M. Clerici *et al., J. Clin. Invest. 91*: 759-65 (1993)). Although the decreased responses to alloantigens and mitogens may be at least partially explained by alteration in relative numbers of CD4 and CD8 cells placed in tissue culture, this defect in responsiveness to soluble antigens is seen even when one studies purified CD4 cells (H.C. Lane *et al., New England J. Med.* 313: 79-84 (1985)). In fact, one of the earliest immune defects associated with HIV infection is this loss of ability to respond to recall antigens, and is often present in patients with normal CD4 counts (H.C. Lane *et al., New England J. Med.* 313: 79-84 (1985)).

The ability of intermittent IL-2 therapy to restore *in vitro* lymphocyte function was determined. As shown in Table 2 and Figure 1, intermittent IL-2 therapy of the present invention was associated with an improvement in blastogenic responses in the reverse order of their probable loss.

Another phenomenon observed in HIV patients is the increased percent of human leukocyte antigen-D related (HLA-DR) positive lymphocytes compared to healthy controls. (A. Landay *et al., AIDS* 4: 479-497 (1990); J.V. Giorgi *et al., Clin. Immunol. Immunopathol* 52: 10-18 (1989)) This represents an increase in the proportion of lymphocytes in the peripheral blood that are activated and presumably terminally differentiated. This increase in HLA-DR is seen primarily in CD8 positive cells, and may be a poor prognostic sign (D. P. Sites *et al., Clin. Immunol. Immunopathol* 38: 161-177 (1986)). The percent of HLA-positive lymphocytes of all patients were found to be elevated prior to treatment.

As shown in Figure 2 and Table 2, the intermittent IL-2 therapy of the present invention leads to a decline in the proportion of cells positive for HLA-DR. This decline in HLA-DR positive cells may represent an IL-2-induced improvement in the aberrant homeostatic mechanisms that are regulating CD8 lymphocyte activation in HIV. These decreased levels are observed even one and two months after completion of IL-2 courses.

Levels of IL-2 receptors in CD4 positive cells and in both CD4 and CD8 cells increased during the intermittent IL-2 therapy. This up-regulation of IL-2 receptors is likely a pharmacologic effect of IL-2, and may explain why some patients had increases in CD4 but not CD8 cells, while other patients had increases in both. The increase in IL-2 receptor-expressing cells also may be responsible for the improvement in blastogenic responses, since such responses are dependent on recruitment of initially unresponsive cells, and such cells, if expressing IL-2 receptors, can respond more easily to IL-2 secreted by the initially activated cells.

Our observations differ from other reports using low doses of recombinant IL-2 or polyethylene glycol (PEG) IL-2 administered subcutaneously, in which no changes in CD4, CD8, HLA-DR, or IL-2 receptor-positive cells were seen (H. Teppler *et al.*, *J. Infect. Dis.* 167: 291-298 (1993); H. Teppler *et al.*, *J. Exp. Med.* 177: 483-492 (1993)). Further, while natural killer (NK) activity has been shown to increase with low doses of IL-2 (H. Teppler *et al.*, *J. Infect. Dis.* 167: 291-298 (1993); M. A. Caligiuri *et al.*, *J. Clin Invest.* 91: 123-132 (1993)), we observed no consistent changes in NK or LAK activity following IL-2 therapy (data not shown).

In accordance with another aspect of the present invention, a composition of matter is provided for administration to a patient who is suffering an immunological impairment or infectious disease. The composition comprises a container suitable for holding a solution to be infused into a patient, and a liquid

preparation comprising an amount of IL-2 in a pharmaceutically acceptable carrier such that the preparation represents an IL-2 dosage of between about 1.8 to about 24 MU. A component is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Ansel *et al.*, PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS, 5th Edition (Lea & Febiger 1990), and Gennaro (ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition (Mack Publishing Company 1990).

The composition of matter further comprises instructions that direct administration of the IL-2 preparation to a patient as a continuous infusion over a period of approximately five days. Such instructions may further direct the administration of a therapy to a patient prior to or concomitantly with the infusing, wherein the therapy targets a specific disease state. Illustrative disease states include HIV infection, mycobacterial infections such as tuberculosis, and fungal infections such as cryptococcal disease. For example, the therapy may be an anti-retroviral therapy such as zidovudine administration.

In accordance with yet another aspect of the present invention, a composition of matter is provided which takes advantage of the activated state of the immune system during the course of IL-2 treatment. In this embodiment of the invention, the composition comprises a container suitable for holding a solution to be infused into a patient, a liquid preparation comprising an amount of IL-2 in a pharmaceutically acceptable carrier such that the preparation represents an IL-2 dosage of between about 1.8 to about 24 MU, and instructions to direct the infusion of the IL-2 preparation and the administration of a retroviral vector to effect an *in situ* transformation of lymphocytes. In contrast to prior art

methods of gene therapy where cells are obtained from the patient, transduced *in vitro*, and infused into the patient, the composition of the present invention allows the direct administration of a retroviral vector to the patient, with the transduction of the cells occurring *in situ*.

In this embodiment of the present invention, the immune system is first activated by administering IL-2, as described above. The IL-2 induces the cells to become activated and to synthesize DNA, which makes them more receptive to transduction by retroviral vectors. A genetically-engineered retroviral vector then is administered directly to the patient. This vector is integrated with the DNA of the patient's own cells, and the administered gene is subsequently expressed. Retroviral vectors that would make a cell resistant to a virus, such as HIV, or that would make a cell able to attack a virus could be introduced into a patient's system by this method. Plasmid DNA can also be used in place of the retroviral vectors, with similar benefits and results seen.

This method is most effective when the vector is administered to the patient when cells are most susceptible to transduction by the vector. Such susceptibility occurs during periods of peak DNA synthesis, which is usually observed during the time period when the IL-2 is being administered.

Figure 4 shows the levels of DNA synthesis occurring *in vivo* in seven patients receiving a 5-day continuous infusion of IL-2 at the above described dosages. Data points were taken prior to IL-2 therapy (PRE), at day 0 of the IL-2 infusions (D/0), at day 5 (D/5) of the IL-2 infusions, and at follow-up visits (F/U). Each peak corresponds to the level of DNA synthesis at day 5 of infusion. This intense *in vivo* T-cell activation seen at day 5 of the IL-2 infusion marks a preferred time to effect T-cell transduction by administering a retroviral vector directly to the patient.

The present invention is further illustrated by reference to the following examples, which illustrate specific elements of the invention but should not be construed as limiting the scope of the invention.

Studies of the effects of intermittent courses of IL-2 on the immune system of immunosuppressed patients were performed. The studies were approved by the National Institute of Allergy and Infectious Disease (NIAID) institutional review board, and all patients provided written informed consent after the risks of the study had been explained.

Patients with HIV infection were eligible for enrollment if they had a CD4 count above 200 cells/mm^3 and had no concurrent opportunistic infections. The cut-off for CD4 counts was selected based on earlier work demonstrating that this group is more likely to respond to immunomodulators than patients with severely impaired immune function.

Because of concerns that IL-2 could lead to enhanced HIV replication, anti-retroviral therapy, primarily zidovudine (AZT), was administered throughout the study. Initial evaluation included a complete history, physical exam, hematology and chemistry profiles, urinalysis, immunologic profiles, p24 antigen levels, and, in some patients, titers of plasma virus (Dewar *et al.*, *Acq. Immune Def. Syndromes*, 5: 822-828 (1992); R. Davey, Jr. *et al.*, *P.N.A.S., USA*, in press) or quantitation of particle-associated plasma HIV RNA using a branched DNA (bDNA) assay (C.A. Pachl *et al.*, Abstract #1247, 32nd INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1992; M.S. Urdea *et al.*, NUCLEIC ACID RESEARCH SYMPOSIUM SERIES No. 24, pages 197-200 (Oxford University Press 1991). Laboratory evaluation was repeated at least monthly.

Example 1. INITIAL TOXICITY TRIALS

Native and recombinant IL-2 was administered to patients by continuous infusion at doses up to 12 MU/day for a three-to-eight-week course.

These dosages of IL-2 were well-tolerated, and transient increases in CD4 counts could be seen (H. C. Lane *et al.*, *J. Biol. Response Mod.* 3: 512-516 (1984)). Bone marrow biopsies obtained at the end of this continuous IL-2 therapy demonstrated a relative lymphocytosis when compared to pre-therapy samples, suggesting that effects of IL-2 were not simply the retrafficking of lymphocytes to the peripheral blood.

Example 2. DOSAGE ESCALATION TRIAL

A dose escalation trial was performed in which 23 patients received a single 21-day or 5-day course of recombinant IL-2 (rIL-2; Chiron) by continuous infusion, at doses ranging from 1.8 million international units (MU)/day to 24 MU/day. All patients received zidovudine (100-200 mg 5id or q4h) beginning at least six weeks prior to the first IL-2 course.

The maximum tolerated dose of recombinant IL-2 when administered for 21 days in combination with zidovudine was found to be 12 MU/day, and when administered for five days in combination with zidovudine was found to be 18 MU/day. Dose-limiting toxicities were similar to those previously associated with recombinant IL-2 therapy alone (J. P. Siegel *et al.*, *J. Clin. Oncol.* 9: 694-704 (1991); M. T. Lotze *et al.*, *Cancer* 58: 2754-2772 (1986)) and included hepatic and renal dysfunction, thrombocytopenia, neutropenia, respiratory distress, and severe flu-like symptoms.

Transient changes were seen in CD4 counts during this phase, but no consistent long-term changes in immune parameters were seen (data not shown). No consistent changes in p24 antigen levels or ability to culture HIV from peripheral blood mononuclear cells were found.

Example 3. INTERMITTENT IL-2 THERAPY

A multiple course study of intermittent IL-2 therapy was performed. Eight patients (six men and two women) received a five-day course of recombinant IL-2 on an inpatient basis by continuous infusion, initially at a dose of 18 MU/day, every eight weeks. Recombinant IL-2 was administered either through a central line IV or a peripheral IV. When peripheral infusions were used, the recombinant IL-2 was placed in 5% dextrose in water (D_5W) containing 0.1% albumin. Zidovudine (100 mg bid) was administered concomitantly. Near the end of the study, didanosine therapy (200 mg bid) was also used in two patients.

The employed dosages of IL-2 generally were well-tolerated and were less toxic than the higher dose regimens typically used in cancer therapy. However, six patients required dosage reduction to 12 or 6 MU/day, primarily because of fever and severe flu-like symptoms. Other toxicities, including metabolic abnormalities, hepatic and renal dysfunction, hypothyroidism, thrombocytopenia, and anemia were seen but were mild and not dose-limiting.

Several parameters were used to evaluate results. Changes in lymphocyte subpopulations (CD4 percent and count, CD8 count, CD4:CD8 ratio, lymphocyte count, and CD3 count) following multiple courses of IL-2 therapy were determined. Flow cytometry was performed on Ficoll-Hypaque-separated peripheral blood mononuclear cells by previously described techniques using monoclonal antibodies to CD3 (T cell), CD4 (helper-inducer T cell), and CD8 (suppressor-cytotoxic T cell) (H. C. Lane *et al.*, *Am. J. Med.* 78: 417-422 (1985)). Values used represent the mean of three pre-study values (Pre-IL-2) and the mean of the two latest values obtained four and eight weeks after the most recent course of IL-2. These results are shown in Table 1. The four-week value tended to be higher than the eight-week value for most patients.

Table 1. Changes in lymphocyte subsets during IL-2 therapy

Pt. No.	Sample	CD4 Percent (% positive)	CD4 No. (Cells/mm³)	CD8 No. (Cells/mm³)	CD4:CD8 Ratio	Lymphocyte No. (Cells/mm³)	CD3 No. (Cells/mm³)
1	Pre-IL-2	20	458	1485	0.31	2303	2096
	Weeks 49/54 (6 doses)	57	2130	1374	1.55	3768	3597
	Percent Change	183	365	-7	401	64	72
2	Pre-IL-2	36	660	879	0.75	1846	1619
	Weeks 52/56 (6 doses)	52	690	516	1.33	1338	1160
	Percent Change	44	5	-41	77	-28	-28
3	Pre-IL-2	14	233	1037	0.22	1690	1407
	Weeks 56/60 (7 doses)	18	765	3383	0.23	4256	4001
	Percent Change	32	229	226	1	152	184
4	Pre-IL-2	30	421	632	0.68	1423	1071
	Weeks 51/56 (7 doses)	31	469	625	0.82	1501	1099
	Percent Change	4	11	-1	21	5	3
5	Pre-IL-2	12	291	1784	0.16	2483	2137
	Weeks 51/55 (6 doses)	13	276	1624	0.17	2195	1865
	Percent Change	7	-5	-9	4	-12	-13
6	Pre-IL-2	19	247	732	0.34	1320	1087
	Weeks 56/60 (4 doses)	28	524	1035	0.71	1919	1681
	Percent Change	47	112	41	110	45	55
7	Pre-IL-2	42	871	776	1.12	2051	1656
	Weeks 26/31 (4 doses)	58	1494	688	2.17	2575	2220
	Percent Change	37	72	-11	95	26	34
8	Pre-IL-2	23	188	397	0.48	817	568
	Weeks 21/26 (3 doses)	25	287	576	0.50	1140	798
	Percent Change	7	53	45	4	39	40

Six of the eight patients showed a consistent and sustained increase of greater than 25% in CD4 number and/or percent (Table 1 and Figure 1). The most dramatic increase was from 20% and 458 cells/mm^3 (mean of 3 values) pre-therapy to 57% and 2130 cells/mm^3 (mean of 2 values) one year later, after completion of six courses of recombinant IL-2 (Figure 1A).

Changes in CD8 number were more variable, and not necessarily concordant with changes in CD4 number. Four patients showed an increase (>25%) in the CD4:CD8 ratio due predominantly to an increase in CD4 cells (Table 1).

The immediate effects of recombinant IL-2 therapy on peripheral blood CD4 count, measured within 24 hours of discontinuation of therapy, were even more dramatic than the long-term effects measured weeks later. Peak CD4 counts of greater than 2000 cells/mm^3 were commonly seen, though these increases were transient (data not shown) and probably reflective of redistribution phenomena.

Changes in immunologic parameters were also determined. To evaluate the ability of intermittent IL-2 therapy to restore *in vitro* lymphocyte function, blastogenic responses to antigens and mitogens were measured. Proliferation assays were performed as previously described (H.C. Lane *et al., Am. J. Med. 78*: 417-22 (1985)), using a 1:800 dilution of PWM, or 3 μ/ml of tetanus toxoid in six day, tetanus toxoid and pokeweed mitogen induced lymphocyte blast transformation assays. Values used represent the mean of three pre-study values (Pre-IL-2) and the mean of the two latest values obtained four and eight weeks after the most recent course of IL-2. The results are shown in Table 2 as net CPM of incorporated [3H]-thymidine. Percent of cells positive for IL-2 receptor (IL-2r) and human leukocyte antigen-D related (HLA-DR) expression were determined by single-color fluorescent activated cell sorter (FACS) analysis using monoclonal antibodies to CD25 (p55 IL-2 receptor) and HLA-DR. FACS analysis was gated for lymphocytes. These results are also presented in Table 2.

Table 2.
Changes in markers of lymphocyte function and activation during IL-2 therapy

Pt. No.	Sample	Tetanus (CPM)	PWM (CPM)	IL-2r (% positive)	HLA-DR (% positive)
1	Pre-IL-2	2003	1757	8	43
	Weeks 49/54 (6 doses)	8479	5981	53	23
	Percent Change	323	240	585	-47
2	Pre-IL-2	118	1762	5	50
	Weeks 52/56 (6 doses)	4250	12321	33	31
	Percent Change	3512	599	509	-38
3	Pre-IL-2	212	1043	5	35
	Weeks 56/60 (7 doses)	100	5760	30	16
	Percent Change	-53	452	500	-56
4	Pre-IL-2	117	1195	8	32
	Weeks 51/56 (7 doses)	100	14216	23	22
	Percent Change	-15	1090	176	-31
5	Pre-IL-2	100	1386	5	47
	Weeks 51/55 (6 doses)	483	1980	8	42
	Percent Change	383	43	60	-11
6	Pre-IL-2	1735	4720	10	32
	Weeks 56/60 (4 doses)	895	5944	25	30
	Percent Change	-48	26	150	-9
7	Pre-IL-2	32054	12568	8	21
	Weeks 26/31 (4 doses)	39708	14041	36	13
	Percent Change	24	12	326	-38
8	Pre-IL-2	121	19103	8	20
	Weeks 21/26 (3 doses)	100	7878	26	19
	Percent Change	-17	-59	206	-3

As shown in Table 2 and Figure 1, IL-2 therapy was associated with an improvement in blastogenic responses in the reverse order of their probable loss. Thus, four of five (80%) patients with absent or poor responses to PWM developed vigorous and consistent responses during the study, and two of the seven non-responders (29%) to the recall antigen tetanus toxoid became consistent responders.

The percent of lymphocytes positive for HLA-DR was found to be elevated (≥20%) in all eight patients prior to study (Table 2). Interestingly, during IL-2 therapy, there was a decline (≥25% of initial values) in the proportion of cells positive for HLA-DR, measured one and two months after completion of IL-2, in 5/8 patients (Table 2 and Figure 2). At the same time, the proportion of cells positive for the IL-2 receptor (IL-2r) (p55) increased progressively (Table 2 and Figure 2) in all patients. In one patient, this increase was minimal (Patient 5, Table 2) and in this patient there was little evidence of improvement in CD4 counts or blastogenic responses. This same patient also showed only a minimal decrease in the proportion of cells positive for HLA-DR.

Based on two-color FACS analysis in three patients, CD8 positive cells were the predominant population positive for HLA-DR prior to study, and were the primary population accounting for the decline in this marker (Figure 2B). IL-2 receptors (IL-2r) increased during IL-2 therapy almost exclusively in CD4 positive cells in patients 1 and 2 (Figure 2B), while patient 3 showed an increase in IL-2r in both CD4 and CD8 cells. This up-regulation of IL-2r is likely a pharmacologic effect of IL-2, and may explain why patients 1 and 2 had increases in CD4 but not CD8 cells, while patient 3 had increases in both.

Figures 1-3 show additional results for the individual patients.

Figure 1 shows changes in CD4 cell count and blastogenic responses to tetanus toxoid and PWM for

patients 1 and 3 during a year of intermittent IL-2 therapy. Arrows indicate the start of each five-day course of continuous infusion IL-2 at an initial dose of 18 MU over 24 hours. Values shown represent results obtained four and eight weeks after each course of IL-2 with the week eight sample drawn immediately before beginning the next round of IL-2.

Figure 1A shows the results for Patient 1, who demonstrated a marked increase in CD4 cells as well as sustained improvement in lymphocyte blast transformation to both stimuli. The last data point is 15 weeks after the sixth course of IL-2 (week 59) at which point the patient's CD4 count remained above 1500 cells/mm^3.

Figure 1B shows the results for patient 3, who demonstrated improvement in lymphoid blast transformation to PWM, but not tetanus toxoid. His CD4 count remained stable until after the sixth course of IL-2, at which time it increased. Didanosine was added to this patient's anti-retroviral regimen at week 38.

Figure 2A shows changes in lymphocyte cell surface expression of IL-2 receptors (CD25) and HLA-DR for patient 2 during a year of IL-2 therapy. Results shown were obtained by single-color FACS analysis using monoclonal antibodies as described in Table 2, on samples obtained four and eight weeks after a course of IL-2. Arrows indicate the beginning of each course of IL-2. A sustained drop in the percentage of HLA-DR positive cells began after the second course of IL-2. The percentage of IL-2 receptor-positive cells increased substantially after four courses of IL-2.

Figure 2B shows two-color FACS analysis of IL-2 receptor (IL-2r) and HLA-DR expression determined on frozen cells of patient 2 obtained prior to IL-2 therapy, and at week 48 (five weeks after the fifth course of IL-2). As shown, the increase in IL-2r in this patient was due to increased expression exclusively on CD4 cells, while the decline in HLA-DR expression was due primarily to a decrease in expression on CD8 cells. Normal values

for CD3+/IL-2r+ cells are 4.4 ± 1.5 %, and for CD3+/HLA-DR+ cells are 8.7 ± 2.9 %.

Figure 3 shows changes in viral markers during IL-2 therapy for patients 2, 3, 4, 6 and 8. Results are shown for samples obtained four and eight weeks after each course of IL-2, as well as those obtained five or six days after (arrows) beginning each five-day course of IL-2. Levels of p24 antigen levels were determined by an immune complex dissociated assay (Coulter Corporation, Hialeah, FL) and particle-associated HIV RNA levels were determined on frozen samples using the bDNA signal amplification assay (Chiron Corporation, Emeryville, CA). C. A. Pachl *et al.*, Abstract 1247, 32nd INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1992; M.S. Urdea *et al.*, NUCLEIC ACID RESEARCH SYMPOSIUM, Series 24, Oxford University Press, pages 1927-200 (1991)

Briefly, virus was concentrated from plasma using a bench top microcentrifuge (Heraeus Contifuge Model 17RS, rotor 3753; 23,500 x g, 1 hour). The resultant virus pellet was lysed with 220 µl of a proteinase K/lithium lauryl sulfate buffer containing target probes complementary to pol gene sequences and then transferred to microwells of a 96-well plate. The RNA target was captured onto the microwell surface via specific capture probes during an overnight incubation at 53°C. The wells were washed and successively hybridized with the branched DNA amplifier (30 minutes), then alkaline phosphatase labeled probe (15 minutes). Finally, a chemiluminescent substrate, dioxetane, was added to each well and the enzyme-triggered light output was measured with a luminometer. The quantity of HIV RNA (reported as RNA equivalents/ml plasma) was calculated based on comparison to a standard curve. Signal was directly proportional to the amount of viral RNA present in the specimen. Not all samples were available at all time-points.

Figure 3A shows that no significant changes were seen in p24 antigenemia during IL-2 therapy. Figure 3B shows that particle-associated plasma HIV RNA tended to

increase transiently immediately after IL-2 therapy (arrows), then returned to baseline. All patients were receiving zidovudine throughout the study. In patient 3, the addition of didanosine at week 38 was associated with a substantial and sustained decrease in plasma particle-associated RNA levels.

No consistent changes in overall viral load in the peripheral blood, as evaluated by serial measurement of p24 antigen levels (Figure 3A) or plasma viremia (data not shown), were detected during multiple-course IL-2 therapy. One patient showed a gradual decline, and two a gradual increase, in p24 antigen levels during a year of therapy. The other five patients remained consistently negative for p24 antigenemia.

Because p24 antigen levels do not appear sensitive to acute changes in plasma viral burden, we assayed frozen plasma from six patients using a recently developed branched DNA assay that quantitatively measures HIV RNA (Figure 3B). See C.A. Pachl *et al.*, *supra*; M.S. Urdea *et al.*, *supra*. In most patients, a consistent increase in particle-associated HIV RNA was noted immediately at the end of a course of IL-2; this increase was not associated with an increase in p24 antigen levels, and was almost always transient, with a return to baseline at the one- and two-month follow-up visits. The clinical significance of this transient burst in viral RNA is uncertain at present, but it likely represents release of HIV following activation of lymphocytes. Alternatively, it could represent a redistribution of virus from lymph nodes or other sites to the blood (G. Pantaleo *et al.*, *Nature 36*: 365-71 (1993)).

In summary, six patients showed a sustained increase in CD4 number and/or percent following IL-2 therapy, with one patient increasing from 458 cells/mm^3 to 2130 cells/mm^3 during the first year of therapy. In addition to increased numbers of CD4 cells, measurements of CD4 function also showed improvement. Four of five initially unresponsive patients developed blastogenic responses to

pokeweed mitogen, and two of seven initially unresponsive patients developed responses to tetanus toxoid. Thus, IL-2 therapy according to the present invention resulted in a decline in the percentage of lymphocytes expressing HLA-DR, and in an increase in the percentage of CD4 lymphocytes positive for the p55 IL-2 receptor. While no changes in HIV load were detected by p24 antigen and plasma viremia assays, a transient but consistent increase in plasma HIV RNA was detected by a new, sensitive branched DNA assay at the end of each infusion.

The patients had three to seven courses of IL-2, and follow-up ranged from 26 to 60 weeks. No patient developed an AIDS-defining opportunistic infection while on study. Accordingly, the use of IL-2 pursuant to the present invention reversed serious immunological abnormalities which are characteristic of HIV infection, especially CD4 cell depletion.

Additional studies demonstrate that intermittent IL-2 therapy enhances the immune system. The objective of an on-going study is to examine the effects of intermittent IL-2 therapy in patients with HIV infection. At the beginning of the study, 31 patients were selected for administration of anti-retroviral therapy with intermittent IL-2 therapy (Group A) and 29 patients were selected for administration of anti-retroviral therapy alone (Group B). In this study, patients receive IL-2 as a continuous infusion approximately every eight weeks. Doses of IL-2 range from 6 to 18 MU over 24 hours for three to five days. Figure 5 shows about one-third of the data that will be obtained from the continuing study. As shown in Figure 5, T cell count increased in patients who received IL-2 treatment, while T cell count decreased in patients who did not receive the IL-2 treatment.

In another study, a cohort of 27 patients with CD4 counts over 200 received IL-2 as a continuous infusion approximately every eight weeks in addition to anti-retroviral therapy. Doses of IL-2 ranged from 6 to 18 MU over 24 hours for three to five days. After six months

of IL-2 treatment, 19 patients (70%) had a 25% or greater increase in CD4 count, 16 patients (59%) had a 50% or greater increase in CD4 count, and 9 patients (33%) had a 100% or greater increase in CD4 count.

A study also was performed with a patient who had idiopathic CD4 lymphopenia. The patient received approximately monthly courses of continuous IL-2 infusion at concentrations ranging from 6 to 18 MU per 24 hours. Figure 6 shows that the T cell count increased during the course of IL-2 treatment.

In conclusion, clinical studies provide evidence for the efficacy of IL-2 therapy in the amplification of immune function.

Example 4. COMBINED IL-2/GENE THERAPY

Interleukin-2 would be given as a continuous infusion at a dose of 6-18 MU/day for a period of six days. At day 5 of the IL-2 infusion the patient would be administered intravenously with a replication-defective, amphotropic retrovirus or with plasmid DNA containing a gene that will render cells resistant to HIV infection. Due to the state of activation of the cells (Figure 4), the genetic information of the retrovirus or the plasmid is incorporated into the genetic information of the cell, rendering that cell resistant to HIV infection.

This method also could be used to broaden the antigen-specific repertoire of the immune system by using recombinant retroviruses or plasmids that contain genetic information for specific antigen receptors.

What Is Claimed Is:

1. The use of an amount of IL-2 in the preparation of an agent for use in a method for treating a disease state characterized by an immunological impairment, where

(A) said amount is sufficient to increase the level of helper/inducer T-cell function in a patient suffering such an impairment

and

(B) said patient is administered said IL-2 in a series of infusions affected intermittently, each of said infusions being continuous over a period of time from 1 day to 2 weeks, and successive infusions being separated by a period of at least 4 weeks.

2. A use as claimed in claim 1, wherein said amount is sufficient to increase CD4 T-cell function in said patient.

3. A use as claimed in claim 1, wherein said amount is sufficient to increase expression of IL-2 receptors in said patient.

4. A use as claimed in claim 1, wherein each of said infusions comprises a dosage of IL-2 of from 1.8 to 24 MU/day.

5. A use as claimed in claim 4, wherein said period of time of each of said infusions is on the order of five days.

6. A use as claimed in claim 1, wherein said disease state is characterized by an infection of said patient by a pathogen against which a cellular immune response is a relevant mechanism for specific immunity for said pathogen in said patient.

7. A use as claimed in claim 6, wherein said disease state comprises a secondary infection of said patient, and wherein said patient has a suppressed immune system.

8. A composition of matter comprising (i) a container suitable for holding a solution to be infused into a patient, (ii) a liquid preparation comprising an amount of IL-2 in a pharmaceutically acceptable carrier such that said preparation represents an IL-2 dosage of between about 1.8 to about 24 MU, and (iii) instructions on infusing a patient with said preparation, said patient suffering an immunological impairment or infectious disease, such that said patient receives a continuous infusion of said dosage over a period of approximately five days.

9. A composition as claimed in claim 8, wherein said instructions further direct administering a therapy to said patient prior to or concomitantly with said infusing, said therapy targeting a specific disease state.

10. A composition as claimed in claim 9, wherein said therapy is an anti-retroviral therapy.

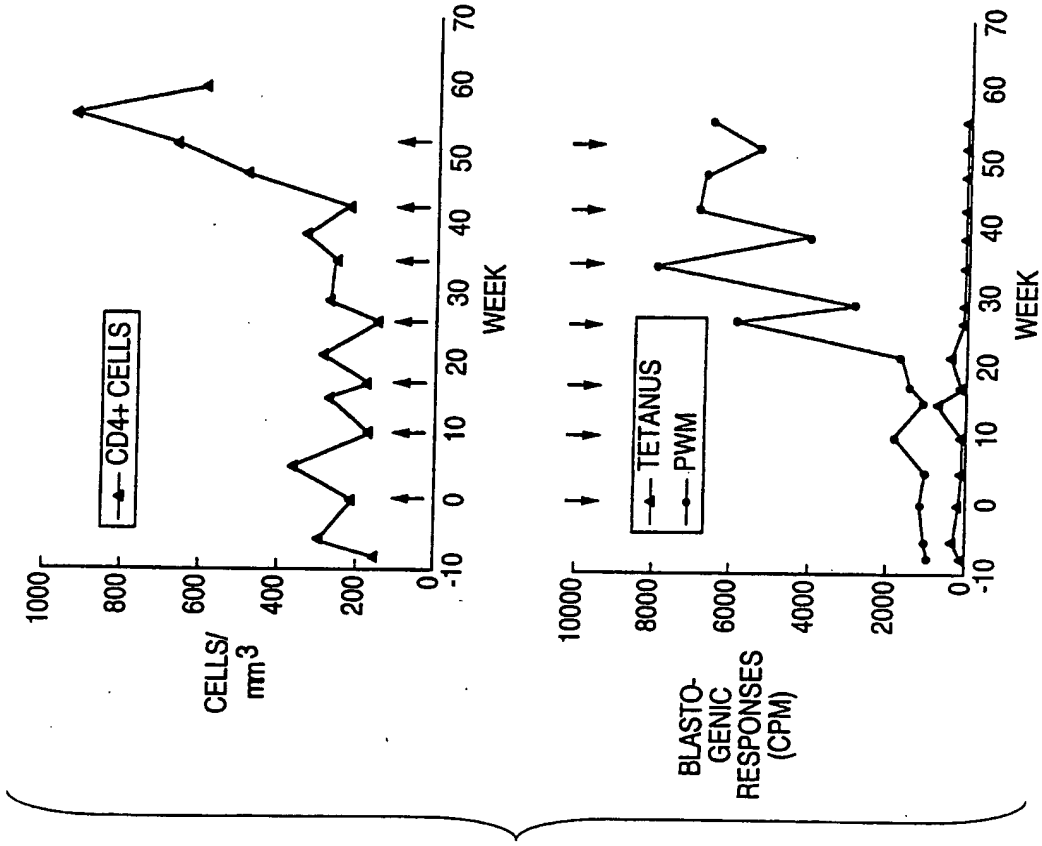
11. A composition as claimed in claim 10, wherein said anti-retroviral therapy comprises administering zidovudine to said patient.

11. A composition of matter comprising (i) a container suitable for holding a solution to be infused into a patient, (ii) a liquid preparation comprising an amount of IL-2 in a pharmaceutically acceptable carrier such that said preparation represents an IL-2 dosage of between about 1.8 to about 24 MU, and (iii) instructions on infusing a patient with said preparation, such that the immune system of said patient is activated, and

administering to said patient a retroviral vector to effect *in situ* transformation of lymphocytes.

12. A composition as claimed in claim 11, wherein said instructions direct that said retroviral vector be administered at the appropriate time during a period of infusion of IL-2.

13. A composition as claimed in claim 12, wherein said instructions direct that said retroviral vector be administered on the 4th to 7th day of IL-2 infusion.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70
WEEK
TETANUS
PWM
BLASTO-GENIC RESPONSES (CPM)
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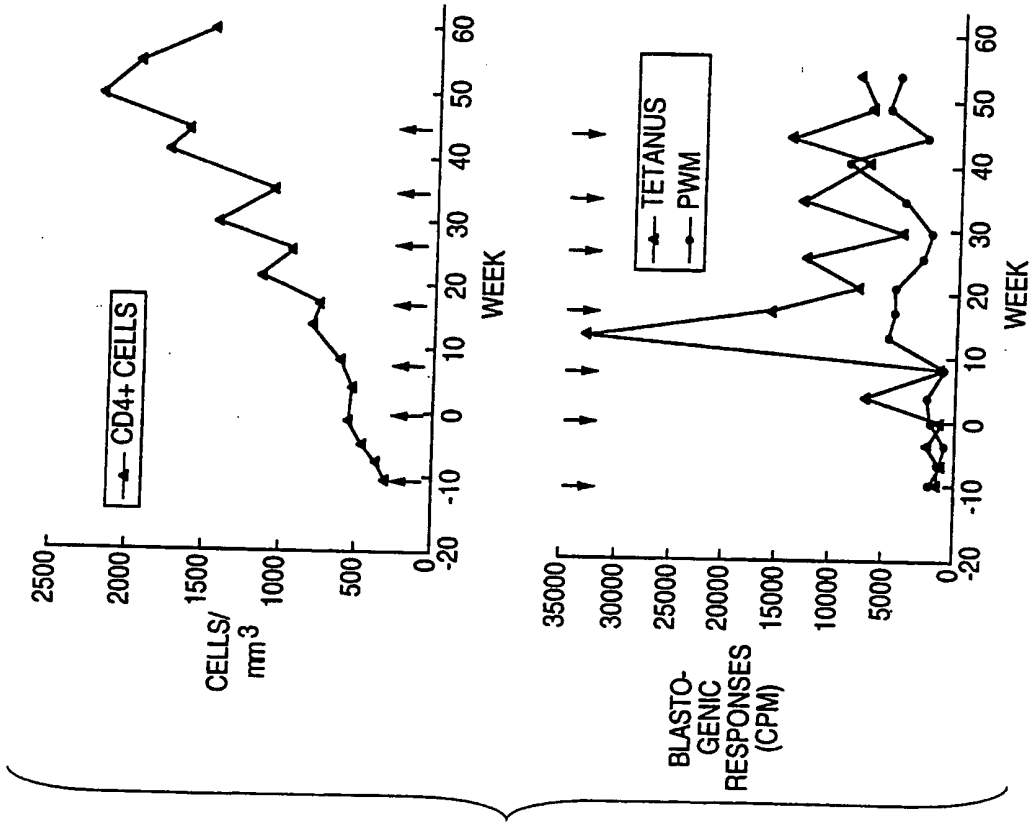
WEEK

FIG. 1A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WEEK
TETANUS
PWM
BLASTO-GENIC RESPONSES (CPM)
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30000
25000
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50
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WEEK

FIG. 1B

FIG. 2A

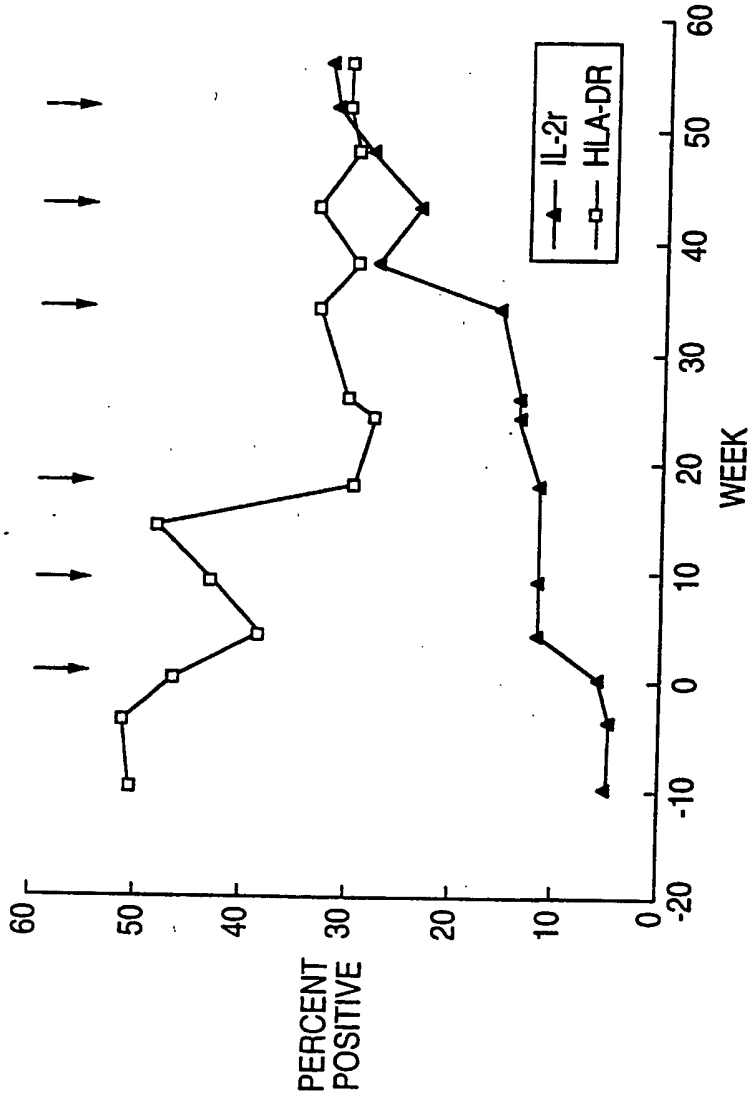
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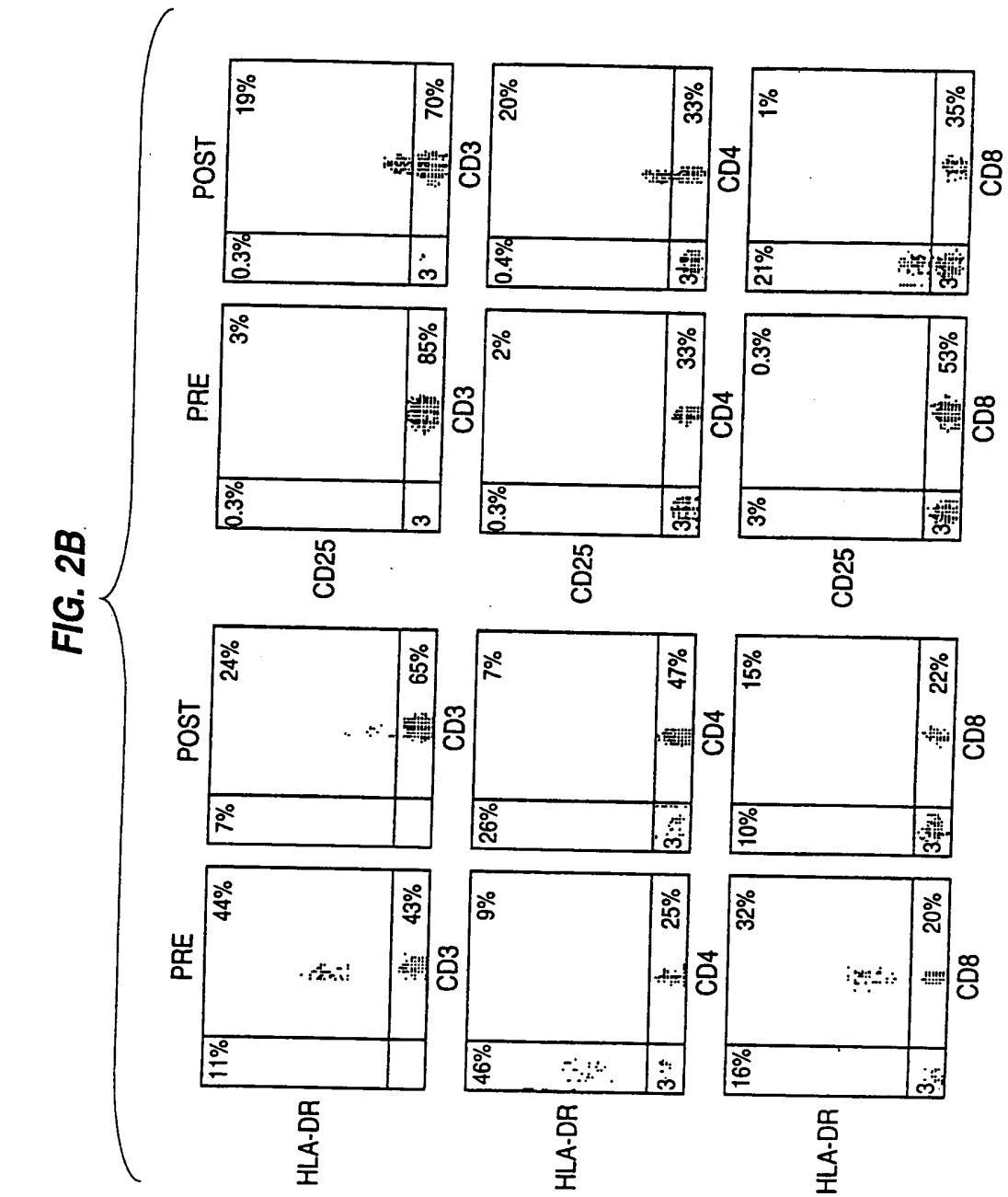
FIG. 2B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26%
7%
47%
16%
32%
20%
10%
15%
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3%
85%
3
19%
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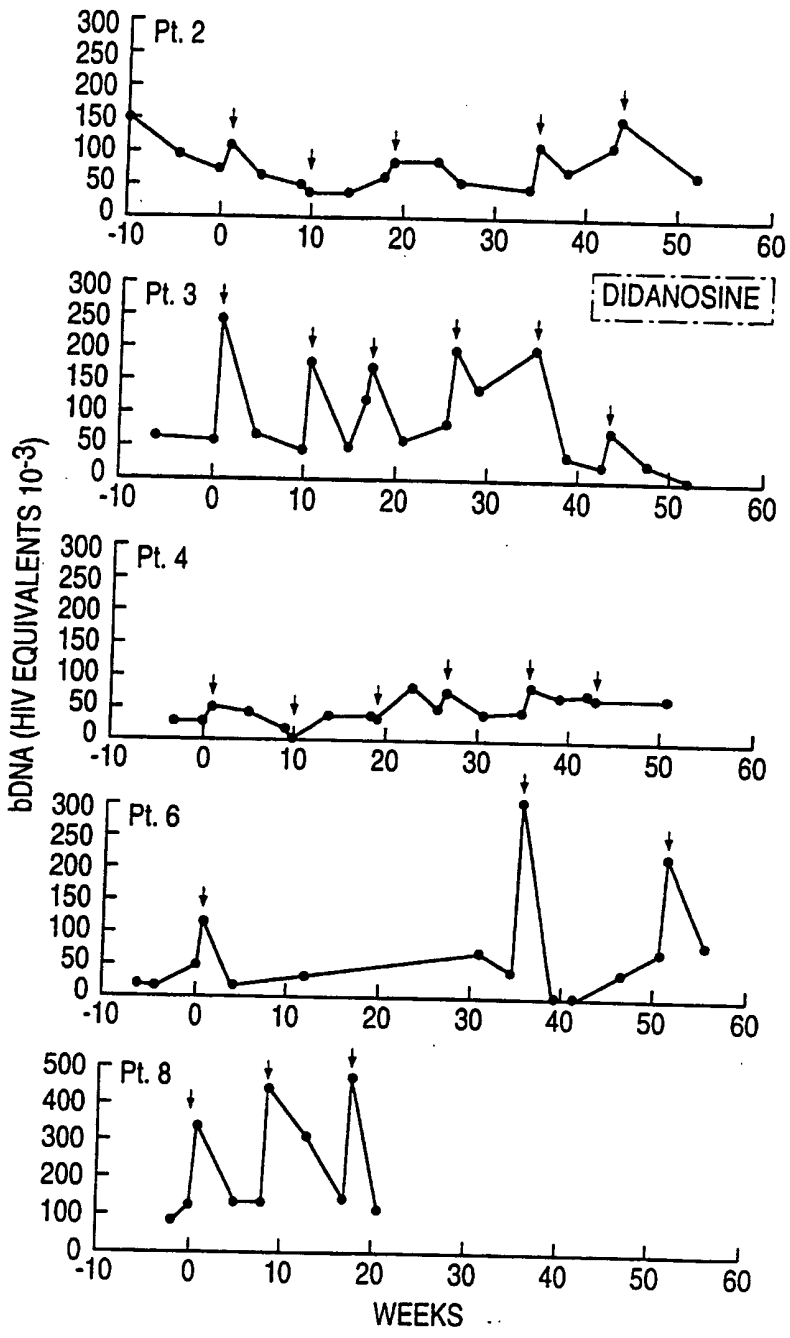
FIG. 3A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Pt. 3
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Pt. 4
Pt. 6
Pt. 8
bDNA (HIV EQUIVALENTS 10^{-3})
WEEKS

FIG. 3B

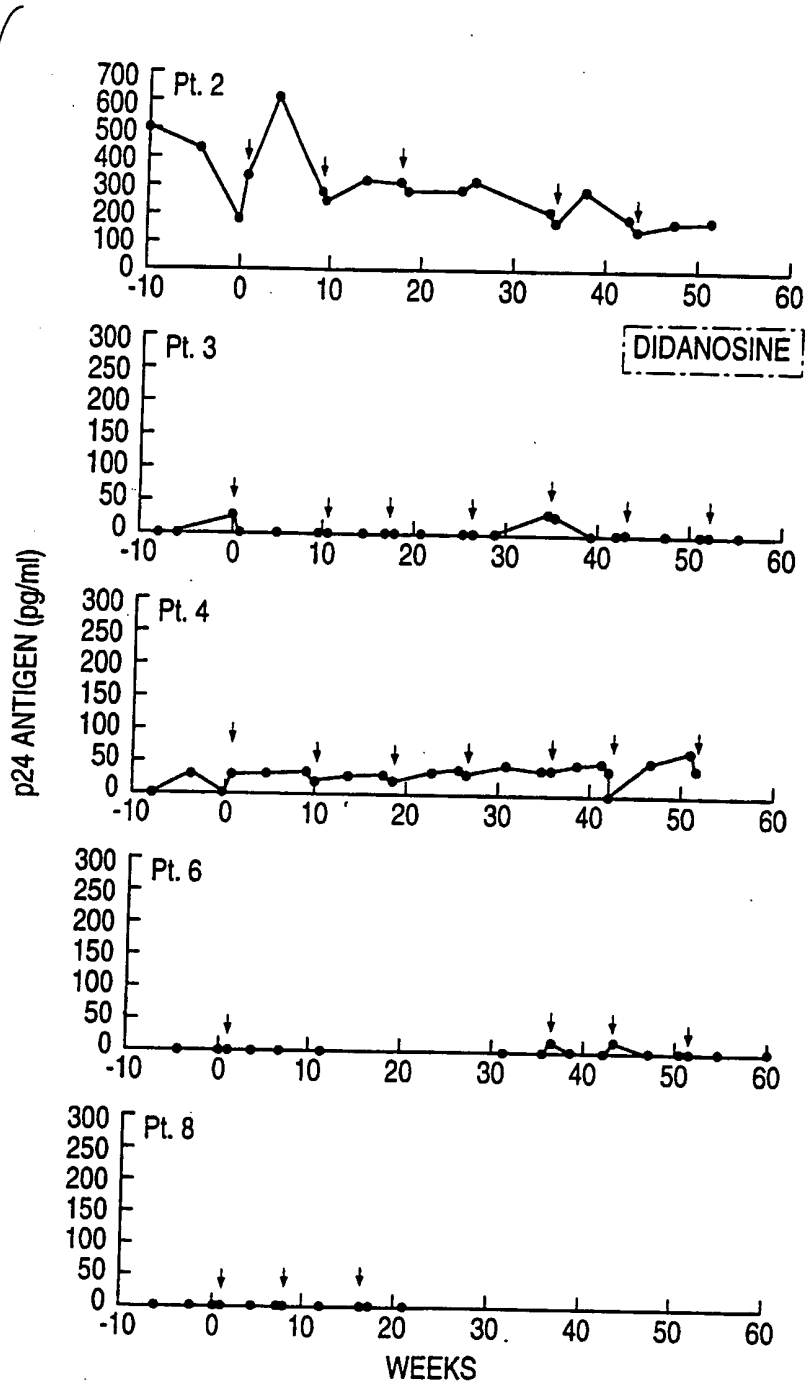
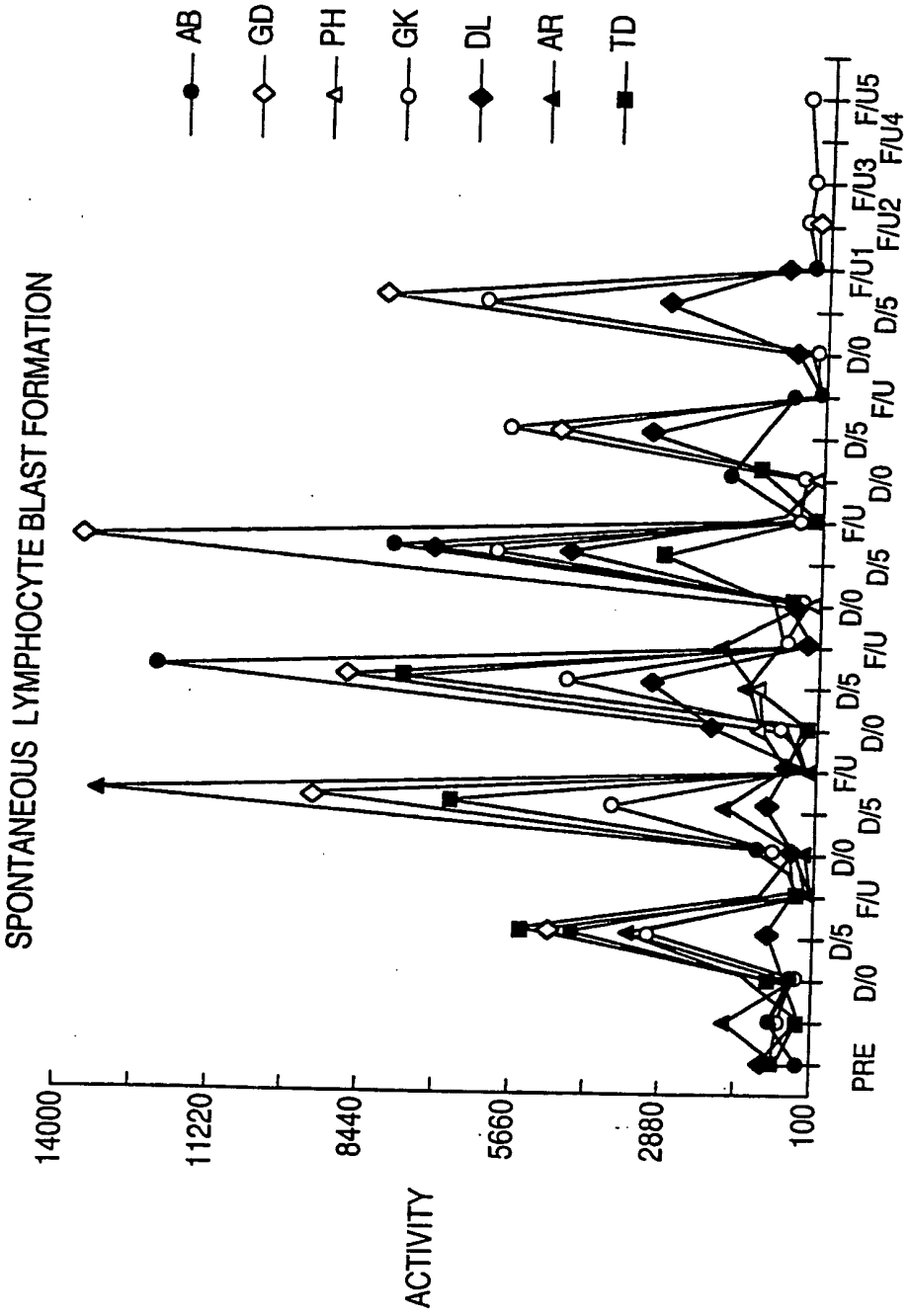


FIG. 4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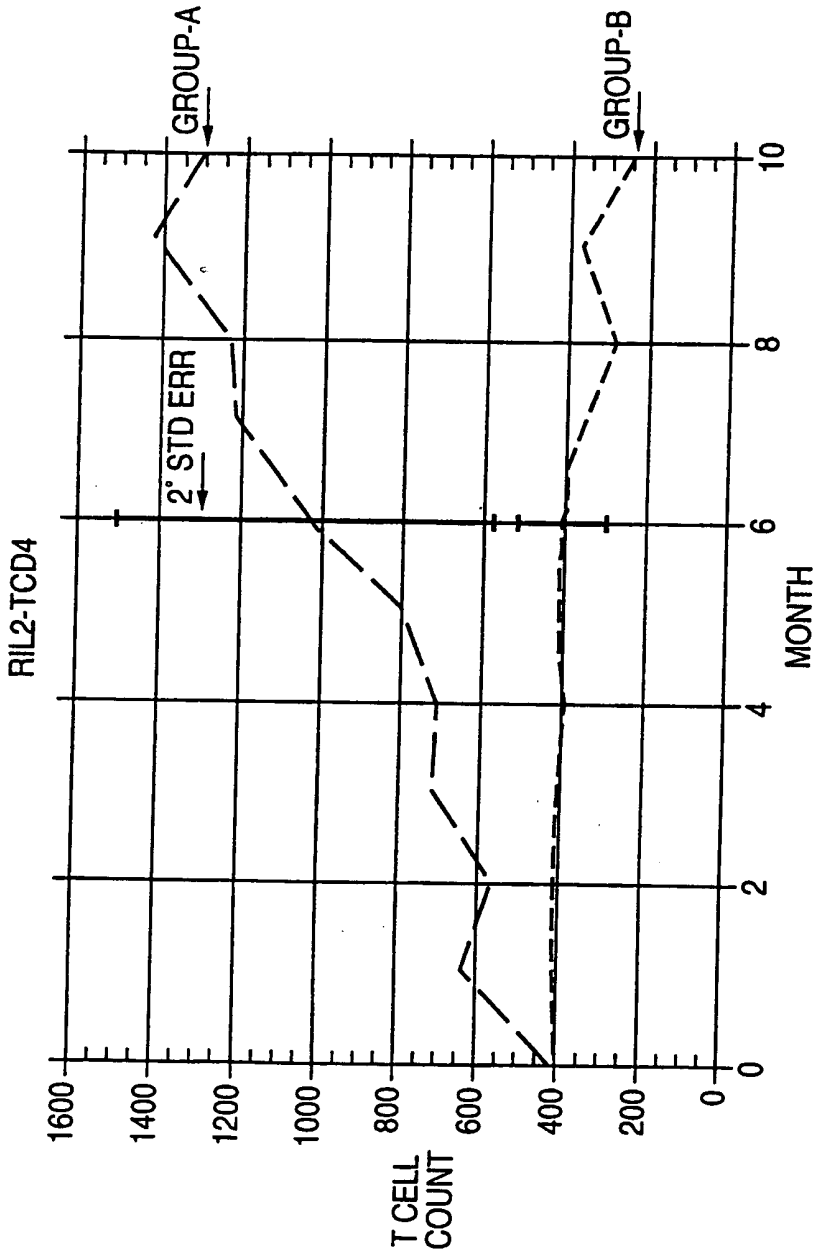
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GROUP-B
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T CELL COUNT
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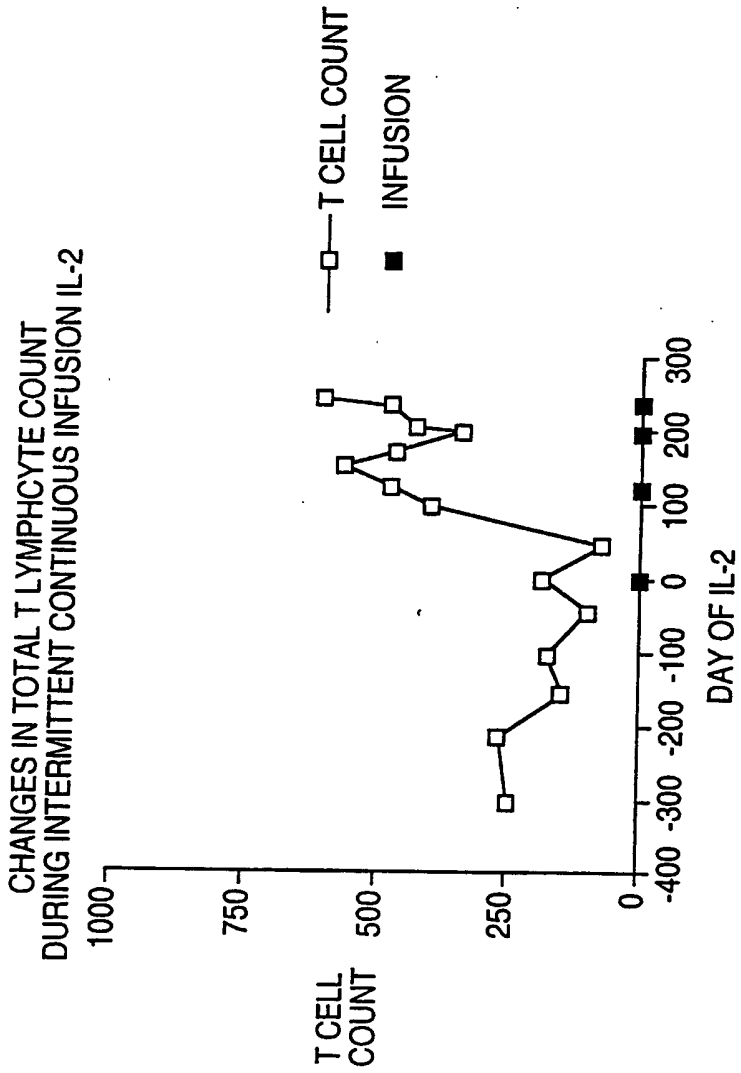
2
4
6
8
10

FIG. 5

FIG. 6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T CELL COUNT
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DAY OF IL-2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/05397

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 343 480 (BIOTEST PHARMA) 29 November 1989 see the whole document ---	1-13
X	EP,A,0 452 598 (ROUSSEL-UCLAF) 23 October 1991 see the whole document ---	1-7
X	EP,A,0 426 521 (ROUSSEL-UCLAF) 8 May 1991 see the whole document -----	1-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 October 1994	27. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Moreau, J

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US 94/05397

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